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Gypchek - The Gypsy Moth Nucleopolyhedrosis Virus Product

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Preface

This handbook is an update of handbook NA-TP-02-92 *"The Gypsy Moth Nucleopolyhedrosis Virus Product"* which was printed in April 1992. This update contains information on virus production, safety evaluations, newly developed commercial carriers, results of efficacy and deposition evaluations, and a copy of the registration label, material safety data sheet, and technical bulletin.

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Contents

Introduction

Gypsy moth

The gypsy moth, *Lymantria dispar* (L.), is a serious defoliator of broadleaved forests in eastern North America. Historically, populations of this insect pest have undergone periodic outbreaks to extremely high densities that resulted in widespread defoliation to an average of 3 million forested acres per year. More recently (1992 through 1996), populations have been declining to levels that result in an average of 1 million defoliated forested acres per year partly due to the rapid spread of the fungus *Entomophaga maimaiga*, which is pathogenic to the gypsy moth. The pest also defoliates trees and shrubs in residential areas and, when infestations are heavy, creates a nuisance to residents. Since the introduction of the gypsy moth and its associated infectious diseases into the Boston area of Massachusetts in 1869, it has spread south and west and continues to spread along the leading edge of infestation at the rate of approximately 12 miles per year (*Figure 1*).

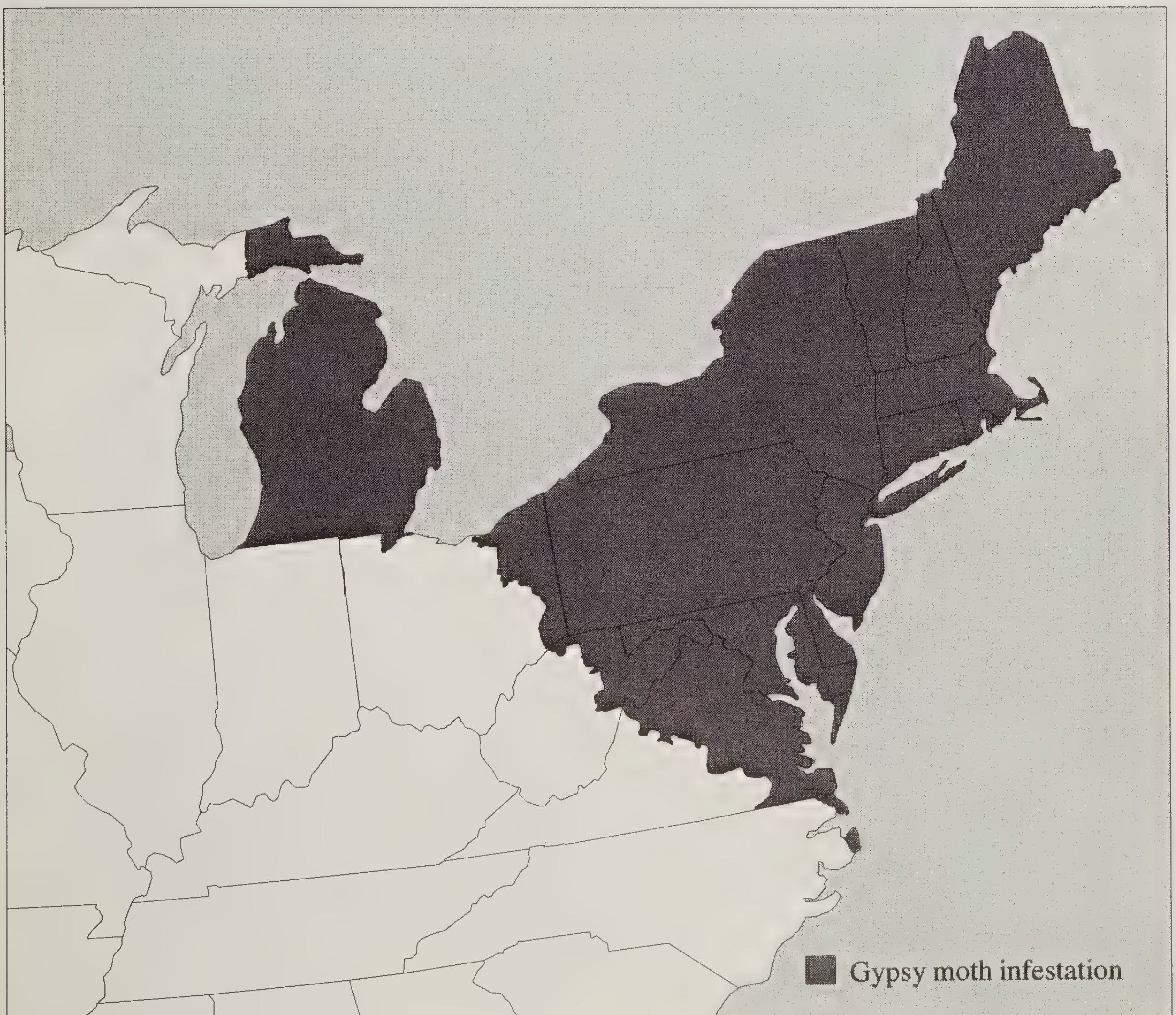

Figure 1. *Distribution of the gypsy moth in the Eastern United States, 1996.*

Virus

Figure 2. *Gypsy moth larvae infected with the nucleopolyhedrosis virus.*

In eastern North America, the gypsy moth is subject to a variety of naturally occurring infectious diseases caused by several kinds of bacteria and fungi as well as a virus (Campbell and Podgwaite 1972). The naturally occurring viral disease (Doane 1970, Campbell and Podgwaite 1972) is often referred to as "wilt" due to the soft, limp appearance of the diseased larvae (*Figure 2*). The disease is caused by a nucleopolyhedrosis virus (NPV) and can reach epizootic (outbreak) proportions as gypsy moth population densities increase. These epizootics result from increased transmission rates, within and between generations of the gypsy moth, as small larvae become infected and die on leaves in the crowns of trees. These larval cadavers disintegrate and serve as inocula for healthy feeding larvae. The larva ingests the viral occlusion bodies (OB) (*Figure 3*) along with the foliage, and the rod-shaped virus particles (virions) are liberated as the polyhedral protein matrix dissolves in the gut. The virions invade through the gut wall and attack internal tissues and organs of the larva, eventually causing a general viral infection. The virus multiplies rapidly in cell nuclei, eventually causing disintegration of internal tissues and death of the larva (*Figure 4*). The entire process takes 10 to 14 days, depending upon the size of the larva, virus dose, and ambient temperature.

Figure 3. *Occlusion bodies of the gypsy moth nucleopolyhedrosis virus under magnification.*

Larvae about to die have a characteristic oily-shiny appearance. Virus-killed larvae typically hang in an inverted-V, turn brownish-black, are fragile to the touch, and often rupture releasing a brownish-black liquid that contains large numbers of occlusion bodies. Larvae killed by the fungus *Entomophaga maimaiga* have a similar appearance except that they retain their normal color and are not fragile to the touch (Hajek 1994). The addition of NPV to the environment at dosages consistent with those used for control of the gypsy moth does not raise NPV levels above those that would occur naturally. Virus transmission also occurs when adult females deposit their egg masses on NPV-

contaminated surfaces (*transovum* transmission); larvae hatching from these contaminated eggs the following spring have a high risk of contracting the disease. The virus will persist at high levels in soil, litter, and on bark for at least 1 year following natural epizootics (Podgwaite *et al.*1979). Virus infection is probably initiated at low gypsy moth densities and as the host density increases the virus spreads due to density-dependent processes (Woods and Elkinton 1987). Birds and mammals have the ability to pass and disperse active gypsy moth NPV (Lautenschlager and Podgwaite 1979), and parasites and invertebrate predators may play a role in the transmitting of gypsy moth NPV within natural populations. Reardon and Podgwaite (1976) found significant positive correlations between the incidence of NPV disease and the incidences of the parasites *Cotesia* (=*Apanteles*) *melanoscelus* and *Parasetigena silvestris*. Further, Raimo *et al.*(1977) showed that *Cotesia* females could transmit NPV from infected to healthy gypsy moth larvae. In many dense gypsy moth populations, the virus kills up to 90 percent of the larvae and reduces populations to levels where they cause only minimal defoliation and tree damage in the following year.

Figure 4. *Gypsy moth virus life cycle: viral occlusion bodies (OB) (A) dissolve in the insect's gut liberating nonoccluded virus (NOV) that enters the midgut (B) and eventually passes through to the hemocoel. There NOV enters hemocytes and other cell types and replicates (C), producing more NOV (D) and OB (E). Cells eventually rupture releasing NOV and OB into the hemocoel. The insect dies (F) 10-14 days after consuming the virus.*

Virus Product Development

Registration

In the late 1950's, the USDA Forest Service began to explore the feasibility of developing the naturally occurring virus as an alternative to chemical insecticides for suppressing gypsy moth populations. In April 1978, after many years of research and development, the gypsy moth nucleopolyhedrosis virus product Gypchek (*Figure 5*) was registered by the U.S. Environmental Protection Agency (U.S.- EPA) as a general use insecticide for aerial and ground application to control gypsy moth. The product has recently satisfied all reregistration requirements established by the U.S.- EPA. A similar product, Disparvirus, was developed in Canada and is in the registration process there (Nealis and Erb 1993). Current labeling (*Appendix A*) does not require that the product be used under Forest Service supervision, although consultation with Forest Service personnel familiar with its use is recommended during the planning phase of a project.

Today, Gypchek is one of two biological insecticides (the other being the bacterium *Bacillus thuringiensis kurstaki*) currently registered for use against gypsy moth. The Hamden strain of the gypsy moth NPV (isolated from a Connecticut population of NPV-killed gypsy moth larvae) is the active ingredient in the currently registered product.

Figure 5. *The gypsy moth nucleopolyhedrosis virus product, Gypchek.*

Identity and Safety

The active ingredient in Gypchek is classified as a member of the genus *Baculovirus* (subgenus A, nuclear polyhedrosis virus).The virus has been shown to represent a complex of 10 closely related genotypic variants (Slavicek and Podgwaite 1991). Based upon biochemical and biophysical analyses, serological and immunological testing, and enzymic analysis of viral DNA, the variants have been shown to be unrelated to human and other mammalian viruses and only distantly related to other insect viruses (USDA 1995). *Appendix B* is the Material Safety Data Sheet for Gypchek.

Toxicological and pathogenicity testing of Gypchek on laboratory animals, wild mammals, birds and fish in support of registration have, with one exception, revealed no effects. That exception is that the product has been shown to be an irritant when applied in massive amounts to the eyes of laboratory rabbits. The irritation was caused by the finely ground insect parts that are a natural component of the product. It is extremely unlikely that any mammalian or avian species would ever encounter such high dosages (irritating levels) of product following application.

The remote possibility that gypsy moth NPV might be related to the arthropod-borne (arbo) viruses and other viruses that infect man has been investigated. In studies carried out at Yale University, all of the known arboviruses were found to be serologically unrelated to gypsy moth NPV. Other viruses, including *Herpes* spp., were also found to be serologically unrelated to gypsy moth NPV (Mazzone *et al.*1976). In addition, extensive testing of NPV on aquatic and terrestrial invertebrates has confirmed the extremely narrow host range of gypsy moth NPV. Specificity testing by Barber *et al.*(1993) and Glare *et al.*(1995) have revealed that the virus is non-pathogenic to beneficial insects.

Production

Gypchek is produced using an *in vivo* (in whole animal) process in a collaborative effort with the Animal and Plant Health Inspection Service (APHIS), Otis, Massachusetts (Bernon *et al.*1991, Podgwaite 1991). Before 1986 Gypchek was a "whole cadaver" product that was difficult to formulate and apply. The current process (*Figure 6*) described below results in the production of a finely ground powder in which the active ingredient is highly concentrated and easily blended into commercial spray additives.

A standard laboratory strain of the gypsy moth (New Jersey) is reared year-round to provide egg masses which are held for 150 days at 6 °C to complete diapause (quiescence). The egg masses are dehaired and the eggs are mechanically placed onto diet in 6 oz. cups. Larvae emerging from eggs are reared for 14 days at 26 °C. When larvae reach early fourth instar, the cups are inoculated with 1 ml of a suspension containing 5 x 10^6 viral occlusion bodies (OB) per milliliter. Larvae are reared on the inoculated diet at 29 °C. Fourteen days after inoculation more than 70% of the larvae are dead and the remainder are moribund.

Figure 6. In vivo *production and processing of gypsy moth nucleopolyhedrosis virus.*

Larvae are harvested into plastic bags and held at -30 °C until they are processed. Frozen larvae are thawed for 24 hours at 4 °C and then blended with water at high speed for 10 seconds to release the occlusion bodies. The crude concentrate is poured through a vibrating mesh screen and then through several layers of cheesecloth to remove hairs and large body parts. The concentrate is then subjected to continuous-flow centrifugation. The solids are removed, layered onto trays, and frozen at -35 °C. The frozen solids are lyophilized (24-36 hours) and finely ground to yield the final product (*Figure 5*), which contains about 15% occlusion bodies.

The final product is subjected to quality assurance testing before packaging and distribution. In general, it takes between 500 and 1000 infected gypsy moth larvae to produce enough Gypchek to treat 1 acre. Under the current Forest Service-APHIS cooperative agreement about 15,000 acre equivalents (AE) are produced annually (1AE = 4×10^{11} OB).

Currently, research is being conducted toward developing enhanced viral strains for cell culture production (Slavicek 1995). So-called *in vitro* (in tissues) production is an alternative to the costly *in vivo* technology and will eventually provide a cleaner product, free of microbial contaminants, that can be easily formulated as either a ready-to-use flowable concentrate or a wettable powder. American Cyanamid Company (Princeton, NJ) is producing limited quantities of a gypsy moth NPV strain that exhibits biological properties that make it amenable to production in large fermenters.

Research and Development

Since its registration, Gypchek has been the subject of intense research primarily aimed at maximizing efficacy through the development of formulations that enhance viral persistence and application systems that efficiently deliver effective doses to target foliage.

Before 1987 – Early Virus Product

The "early" Gypchek product was applied twice, 7-10 days apart, using conventional aircraft delivery systems. It was tested at various dosages and volumes, and applied in the evening and morning using various types of aircraft and nozzles. The early field dosage was 1×10^{11} occlusion bodies (OB) per acre per application. Tank mixes contained a sunscreen (to protect the virus from ultraviolet light), a feeding stimulant (to increase gypsy moth consumption of treated foliage), and a sticker (to adhere spray droplets to foliage). Efficacy results from testing the "early" product were often inconsistent, due to several factors: physical characteristics of the product, low activity, marginal dosages, inadequate sunscreen in the tank mix, and poor timing of application.

The Maryland Integrated Pest Management (IPM) Gypsy Moth Project conducted by the USDA Forest Service sponsored a series of yearly (1983 through 1987) field evaluations of various formulations, sunscreens (e.g., folic acid, Dipel 6L carrier), dosages, rates and numbers of applications of Gypchek (Reardon *et al.* 1987). In 1986, a lignosulfonate product Orzan LS (ITT Rayonier Inc., Seattle, WA), which is a by-product of the tree-pulping process, was demonstrated to be an effective sunscreen in laboratory assays (Podgwaite and Shapiro 1986). It displayed strong absorbance of ultraviolet light in the range of 290-340 nm, those wavelengths considered most deleterious to NPV. A Gypchek-Orzan formulation was field tested using ground hydraulic equipment against gypsy moth populations in eastern Maryland. Results indicated that larval

mortality was significantly higher in treated than in untreated woodlots. Most encouraging was the extended persistence of Gypchek activity — foliage collected 14 days after treatment still retained sufficient activity to kill 25% of larvae in a laboratory bioassay.

Figure 7. *Aerial application of Gypchek to manage gypsy moth populations on the George Washington National Forest in Virginia.*

1987 to 1989 – Improved Virus Product

In 1987, a new Gypchek tank mix (*Table 1*) was evaluated in northern and eastern Maryland as part of the Maryland IPM Gypsy Moth Project. This improved tank mix contained fewer inert ingredients (to eliminate nozzle clogging), more active ingredient, and Orzan LS to prolong virus activity on foliage. Results of these tests were encouraging with greater than 98% reduction in egg masses in northern Maryland and 80% reduction in egg masses in eastern Maryland (Podgwaite *et al.* 1992a). This tank mix was also evaluated on similar population densities in the mountainous terrain of the George Washington National Forest in Virginia (*Figure 7*). Results were again positive with a greater than 94 percent reduction of egg masses in five of six treated plots. Defoliation in the untreated plots averaged 67 percent compared with only 22 percent in the Gypchek-treated plots (Podgwaite *et al.* 1992b).

Several factors contributed to the effectiveness of the 1987 and 1988 Gypchek treatments: (1) the tank mix itself and its attributes as previously described; (2) a lyophilized Gypchek powder prepared from an aqueous extract of larval cadavers; (3) two applications 3 days apart allowed more active NPV to be continuously available to the target insect for a 5- to 6-day period and (4) favorable weather conditions during and immediately after application (Reardon and Podgwaite 1994).

1989 to 1992 – Modified Application Parameters

Standard tank mix — The standard tank mix of Gypchek consisted of molasses and lignosulfonate (Orzan LS) at 2 gal/acre/application, and required two applications 3 days apart. These parameters coupled with a limited supply of product, high production costs, and increased demand led to a series of field studies designed to maximize efficacy while minimizing application costs. These studies were conducted over 5 years (1989-1993) as part of the Appalachian Integrated Pest Management (AIPM) Gypsy Moth Demonstration Project by Federal, State, and county agencies, in 38 counties in Virginia and West Virginia (Reardon 1991).

In 1989, two studies were conducted in Virginia. One was in areas of low-density populations (30 to 100 egg masses per acre) on the George Washington National Forest to evaluate the standard tank mix of Gypchek applied twice at 2 gal/acre and 5×10^{11} OB/acre. The other was on the Shenandoah

Table 1. *Gypchek tank mixes for aerial and ground application*

Aerial Application

Standard Tank Mix

Ingredient: 1987-1992	Ingredient: Current	Amount per 3.9 liters (1 gal.)
Orzan LS[1] (ITT Rayonier, Inc., Seattle, WA)	**Lignosite AN** (Georgia-Pacific, Bellingham, WA)	227 g (0.5 lb.) (6% w/v)
Pro Mo Liquid Supplement[2] (Southern States Cooperative, Inc., Richmond, VA)	**MO-MIX**[2] (Southern States Cooperative, Inc., Richmond, VA)	470 ml (16 fl. oz.) (12.5% v/v)
Rhoplex B60A (Rohm & Haas Co., Philadelphia, PA)	**Bond**[3] (Loveland Industries, Greeley, CO)	77.6 ml (2.5 fl. oz.) (2% v/v)
Nonchlorinated Water (pH 5.0-8.0)	**Nonchlorinated Water** (pH 5.0-8.0)	3.24 liters (110 fl. oz.) (85% v/v)
Gypchek	**Gypchek**	100-250 billion OB

Carrier 038 Tank Mix

Ingredient	Amount per 3.9 liters (1 gal.)
Carrier 038[4]	3.60 liters (122 fl. oz.) (95% v/v)
Nonchlorinated water	0.19 liters (6 fl. oz.) (5% v/v)
Gypchek	200-500 billion OB

[1] *This Lignosulfonate powder is no longer in production by ITT Rayonier*
[2] *Mixture of condensed molasses and corn extracts*
[3] *Synthetic latex*
[4] *Abbott Laboratories, Chicago, IL*

Ground Application

Ingredient	Amount per 3.9 liters (1 gal.)
Lignosite AN	227g (0.5 lb.) (6% w/v)
Bond	77.6 ml (2.5 fl. oz.) (2% v/v)
Nonchlorinated water	3.71 liters (125 fl. oz.) (98% v/v)
Gypchek	10 billion OB

National Park to evaluate one (1×10^{12} OB/acre) versus two (each 5×10^{11} OB/acre) applications of the standard tank mix of Gypchek. The results from one application were not encouraging, although the test was compromised by both rainy weather during and immediately after application and the collapse of gypsy moth populations in the untreated plots. In the low-density plots, egg mass populations were reduced 92% by Gypchek (i.e., an overall 55-fold increase in egg mass density in untreated plots compared with 4.5-fold increase in Gypchek plots). These results represented the initial evaluation of aerially applying Gypchek against low-level gypsy moth populations (Podgwaite *et al.* 1993).

In 1990, field evaluations were conducted in central Pennsylvania using the Gypchek standard tank mix at three dosages: 5×10^{11} OB/acre, 2.5×10^{11} OB/acre, 1.25×10^{11} OB/acre. The results were as follows:

Dosage (OB/acre)	Population Reduction %	Foliage Protection %
5×10^{11}	84	50
2.5×10^{11}	85	46
1.25×10^{11}	46	47

The favorable results using the mid-range dosage provided the basis for additional evaluation of similar doses.

Modified tank mix — In Virginia in 1991, Gypchek in a modified tank mix (higher concentrations of Orzan and molasses) was applied twice at 2×10^{11} OB/acre (40% of the standard dosage of 5×10^{11} OB/acre) and 0.5 gal/acre (25% of the standard volume of 2.0 gal/acre). Results were compared with a single and double application of the standard tank mix at a dosage of 5×10^{11} OB/acre in 2 gal/acre/ application. Results showed that egg mass populations were reduced 68 percent by the standard double applications, 66 percent by the standard single application, and 61 percent by the modified tank mix double application. Defoliation in all the Gypchek treatments averaged 25 percent compared with 80 percent in the untreated plots. Defoliation in the standard treatment plots (two applications) was marginally lower than in either the standard (one application) or the modified Gypchek treatment plots. These results indicated that the 2×10^{11} OB/acre/application dose of the modified tank mix of Gypchek would be operationally acceptable.

1992 to 1996 – Commercial Formulations and Carriers

In 1992, two applications of the modified tank mix (10 percent Orzan, 25 percent molasses and 2 percent sticker) at 2×10^{11} OB/acre/application in 0.5 gal/acre/application was pilot tested in Pennsylvania. Three 200 acre plots (each were paired with a corresponding untreated plot) were treated, but interpretation of the results was complicated by an area-wide collapse of the gypsy moth population. Collections of larvae before and after treatment indicated that the dose was adequate for desired mortality (greater than 60 percent) but that the rate of 0.5 gal/acre was probably marginal for the desired droplet distribution on foliage with this reduced dose.

In 1992, the USDA Forest Service collaborated with American Cyanamid Company in the development and evaluation of a commercial wettable powder formulation of Gypchek, and with Entotech, Inc. (Davis, CA) in the development and evaluation of an aqueous flowable spray carrier for Gypchek, to replace the standard lignosulfonate-molasses tank mix. After numerous laboratory and spray tower evaluations of

efficacy and weatherability, one ready-to-use formulation from each company was selected for field testing. Field tests were conducted in Pennsylvania in cooperation with the Pennsylvania Bureau of Forestry and the USDA Agricultural Research Service. The treatments were (1) standard Gypchek tank mix applied twice at 5×10^{11} OB/acre and 2 gal/acre; (2) American Cyanamid wettable powder formulation of Gypchek applied twice at 5×10^{11} OB/acre and 1 gal/acre; (3) American Cyanamid formulation tank-mixed with an optical brightener (0.5% Blankophor BBH), an enhancer of viral activity manufactured by Burlington Chemical Co., Burlington, NC, applied twice at 5×10^{11} OB/acre and 2 gal/acre; (4) Gypchek in the Entotech spray-carrier, applied twice at 5×10^{11} OB/acre in 1 gal/acre; and (5) untreated.

The evaluation of results was again complicated by a natural area-wide NPV epizootic. However, treatment effects were evident for all formulations from the mortality of early stage larvae (Reardon and Podgwaite 1994).

In 1993, the two ready-to-use products were reevaluated in separate projects in Michigan (Onken 1996). One project tested the efficacy of the American Cyanamid ready-to-use formulation with and without brightener. There were five treatments: (1) two applications (1 gal/acre/application) of 2×10^{11} OB/acre with; and (2) without the brightener; (3) two applications of 2×10^{10} OB/acre with the brightener; (4) one application of 4×10^{10} OB/acre with the brightener; and (5) untreated.

The second project evaluated the Entotech spray carrier at two dosages (2×10^{11} OB/acre and 5×10^{11} OB/acre) and 1 gal/acre, the standard tank mix at 5×10^{11} OB/acre and 2 gal/acre. All treatments were applied twice.

None of the American Cyanamid treatments protected foliage nor reduced populations when compared with untreated. These negative results were not anticipated based on the favorable results from the previous year's field test. Formulation "changes" just before field application were part of the problem as determined by post spray laboratory bioassays. Both Entotech treatments provided foliage protection and population reduction compared with the standard tank mix. Therefore, two applications of the Entotech tank mix at 2×10^{11} OB/acre and 1.0 gal/acre was recommended for use in the 1994 Federal and State Cooperative Suppression Program.

In 1994, additional field trials were conducted in Virginia using the Entotech tank mix. Three treatments were evaluated: (1) two applications, each at 2×10^{11} OB/acre in 0.5 gal/acre, (2) two applications, each at 2×10^{11} OB/acre in 1.0 gal/acre, and (3) untreated. Neither population levels based on egg mass counts nor defoliation differed significantly between the treated and untreated plots. Treatments reduced populations based on collections of early stage larvae and comparison with untreated plots. However, results were severely compromised by three major factors: (1) 80-100% foliage expansion at the time of treatment, (2) the majority of larvae were in late second and early third instars, and (3) due to cold, rainy weather, applications had to be staggered over a period of 7 days. However, results from 1993 and 1994 studies still supported the continued use of a double application of the Entotech tank mix, although additional evaluation was needed to determine whether 0.5 gal/acre was as effective as 1.0 gal/acre.

In 1995, field tests were conducted in southwestern Virginia. There were five treatments: (1) Entotech tank mix, applied twice at 2×10^{11} OB and 1.0 gal/acre and (2) 0.5 gal/acre, (3) the standard tank mix applied twice and at 2×10^{11} OB and 2 gal/acre, (4) the Entotech tank mix applied once at 4×10^{11} OB and 1 gal/acre, and (5) untreated. Results indicated that the Entotech tank mix of Gypchek

applied twice at either 1.0 gal/acre or 0.5 gal/acre provided a level of efficacy comparable to the standard tank mix applied twice at 2 gal/acre. These results supported the recommendation of the Entotech tank mix (instead of the standard tank mix) for use against gypsy moth, although further evaluation of the number of applications, the dose, and the rate of application were needed.

In 1996, a pilot test of the Entotech tank mix applied once at 1×10^{12} OB/acre in 1 gal/acre was conducted in Maryland and West Virginia. This increased dose was used to compensate for the need for two applications (3 days apart), which is an undesirable option for operational use. The results indicated that the treatments provided significant larval reduction when compared with the untreated plots. Once again, however, results were complicated by a general population collapse in the treated and untreated plots.

Also, in 1996 an American Cyanamid produced *in vitro* strain of the gypsy moth nucleopolyhedrosis virus was tank mixed at a low dose with the brightener and applied to individual trees using ground application equipment. Although the results were favorable, an additional year of field testing is recommended before conducting aerial applications on small replicated plots. This 1996 test followed several years of testing various strains of gypsy moth NPV and spray adjuvants with ground hydraulic equipment for the purpose of developing protocols for spraying small acreages and individual trees in yards (Webb *et al.* 1990, 1993, 1994a, 1994b, 1996). These tests, conducted cooperatively with USDA-ARS scientists, have shown that it is possible to lower the current recommended dose rate for ground application (1×10^{12} OB/ 100 gal/acre) tenfold and still maintain comparable efficacy by including brightener in the tank mix as an enhancer of viral activity.

The acres that have been treated with Gypchek during field experiments, pilot tests, and suppression and eradication programs from 1963 through 1996 are presented in *Figure 8.*

Deposition

To provide supporting data for a specific level of efficacy, foliage deposition evaluations have been conducted on companion plots not evaluated for efficacy. This was necessary due to the uncertainty concerning the impact on efficacy of UV-protective dyes added to the tank mix to allow quantification of deposit. In the earliest deposit evaluations, it was determined that the standard tank mix at 2.0 gal/acre and the Entotech tank mix at 1.0 gal/acre provided similar deposition on oak foliage. However, deposit from the standard tank mix persisted longer after rainfall than did deposit from the Entotech tank mix. In 1993, the results for deposition on oak foliage in Michigan for the Entotech and standard tank mixes indicated an average volume median diameter (VMD) of 382 microns and 8.3 drops/cm^2. For the 1994 spray trials in Virginia, the average VMD was 280 microns and 4.1 drops/cm^2. The differences in deposit per unit area of leaf surface (8.3 and 4.1 drops/cm^2) was attributed in part to the minimal (less than 25 %) leaf expansion in 1993 and maximum expansion (80-100 %) in 1994.

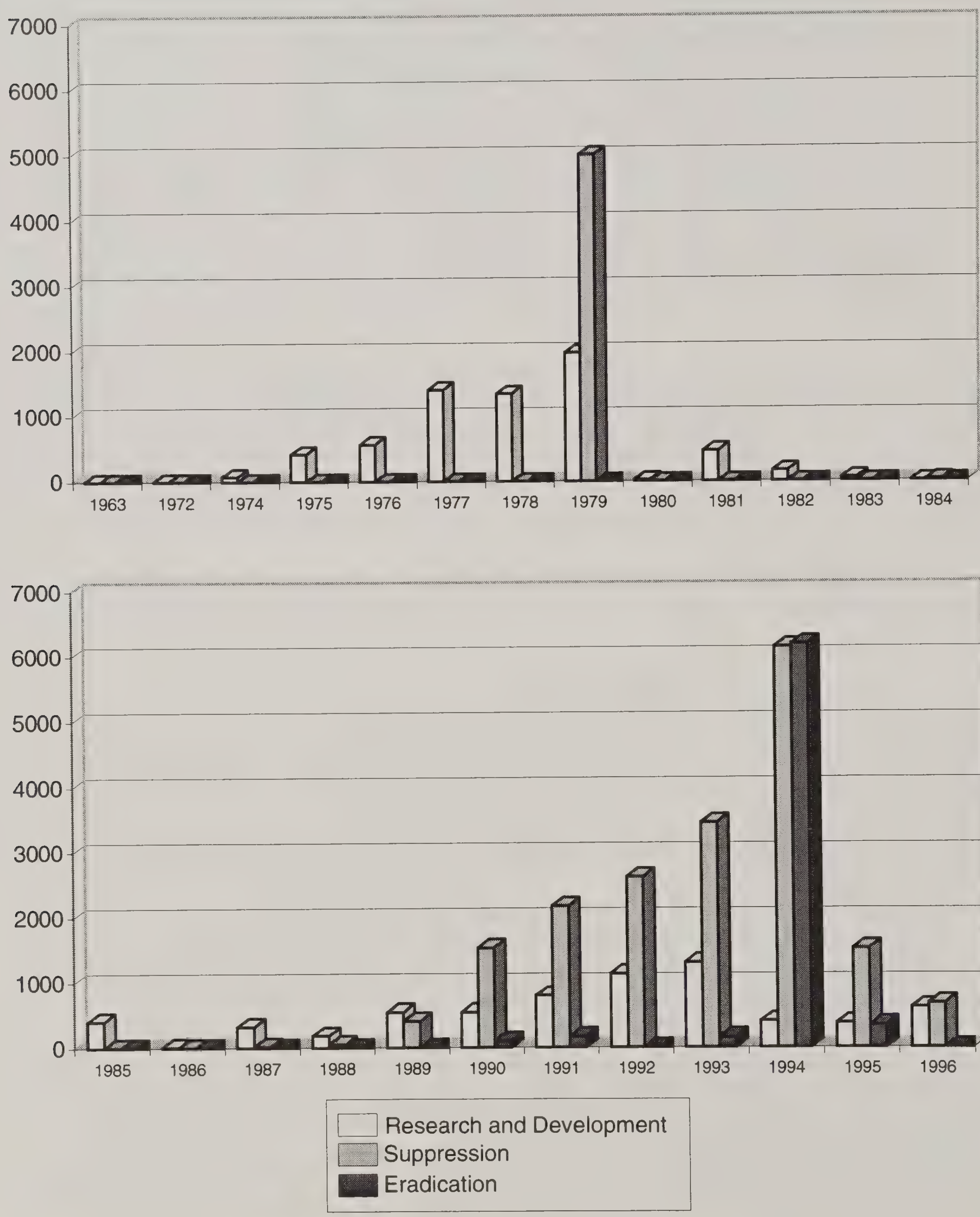

Figure 8. *Acres treated with Gypchek, 1963 - 1996.*

Recommended Tank Mixes for Operational Programs

Two tank mixes are recommended for operational use: the standard tank mix consisting of molasses, a lignosulfonate, a sticker, and water; and the Entotech tank mix consisting of Carrier 038 (now marketed by Abbott Laboratories, N. Chicago, IL), a sticker, and water (*Table 1*). The current recommendations for aerial suppression programs are (1) two treatments 3- days apart of the standard tank mix, each applied at 2×10^{11} OB/application and 2 gal/acre or (2) one or two treatments 3 days apart of the Entotech tank mix, each applied at 2×10^{11} OB or one applied at 4×10^{11} OB and 1.0 gal/acre (*Appendix C*). For aerial eradication programs, the recommendations are (1) two or more treatments 3- days apart of the standard tank mix, each at 5×10^{11} OB and 2.0 gal/acre, or (2) two or more treatments 3- days apart of the Entotech tank mix each at 5×10^{11} OB and 1.0 gal/acre.

For ground hydraulic treatments, pending further tests with brightener the recommendations are (1) one application of a modified tank mix (*Appendix C*) at 1×10^{12} OB and 100 gal/acre for woodlots, roadsides, and small acreages and (2) one application of 15-25 gal of the same tank mix for individual trees in yards.

Gypchek is especially active against smaller larvae and it is recommended that applications be made as soon as hatch is complete, all larvae are off the egg masses and actively feeding, and the majority of the larvae are in the late-first instar. Since Gypchek must be ingested to be effective, leaf expansion should be at levels consistent with larval development (e.g., white oak at least 20% expanded).

Conclusions

The successful field trials with the commercially produced Carrier 038 and Gypchek and the environmental concerns over the effects of broad-spectrum insecticides on nontarget organisms have again stimulated commercial interest. The American Cyanamid effort to produce a strain of nucleopolyhedrosis virus *in vitro* and its subsequent field testing using ground application is promising. Additional field testing of this product applied at a reduced field dose and tank mixed with an optical brightener offers the possibility of a more cost effective product. Forest Service Research will continue to identify, develop and test both natural and genetically engineered strains of gypsy moth NPV that are more virulent than the Connecticut strain that is used in the current Gypchek product. Also, efforts will continue toward developing new, ready-to-use spray adjuvants that will extend NPV persistence on foliage.

Gypchek tank mixed as the standard or with the Entotech carrier 038 is recommended for use against moderate to high density gypsy moth population (300-5,000) egg masses per acre. However, testing in low level populations (less than 100 egg masses per acre), though encouraging, has been limited. Therefore, future field efforts will focus on the evaluation of Gypchek against low density populations (less than 100 EM/acre) as a component tactic in an integrated pest management (IPM) approach to slow the spread of the gypsy moth. Also, additional effort is needed to more fully evaluate the effectiveness of Gypchek for use in eradication programs, where the need is to reduce a population to undetectable levels.

References

Barber, K.; Kaupp, W.; Holmes, S. 1993. Specificity testing of the nuclear polyhedrosis virus of the gypsy moth, *Lymantria dispar* (L.). The Canadian Entomologist 125:1055-1066.

Bernon, G.; Tardiff, J.; Hansen, R.; Podgwaite, J.D.. 1991. Production of gypsy moth nuclear polyhedrosis virus (NPV). Proceedings of the U.S. Department of Agriculture Interagency Gypsy Moth Research Forum 1991. p. 30. USDA Forest Service General Technical Report NE-167, Radnor, PA.

Campbell, R.W.; Podgwaite, J.D. 1972. The disease complex of the gypsy moth. I. Major Components. J. Invertebrate Pathology 18:101-107.

Doane, C. 1970. Primary pathogens and their role in the development of an epizootic in the gypsy moth. J. Invertebrate Pathology 15:21-23.

Glare, T.; Newby, E.; Nelson, T. 1995. Safety testing of a nuclear polyhedrosis virus for use against gypsy moth, *Lymantria dispar*, in New Zealand. Proceedings of the Forty Eighth New Zealand Plant Protection Congress. August 8-10, 1995. p. 264-269.

Hajek, A. 1994. Field identification of the gypsy moth nuclear polyhedrosis virus (NPV). USDA Forest Service NA-PR-01-94. Radnor, PA.

Lautenschlager, R.; Podgwaite, J. 1979. Passage rates of nucleopolyhedrosis virus by avian and mammalian predators of the gypsy moth. Environmental Entomology 8:210-214.

Mazzone, H.; Tignor, G.; Shope, R.; Pan, I.; Hess, W. 1976. A serological comparison of the nuclear polyhedrosis virus of the gypsy moth and European pine sawfly with arthropod-borne and other viruses. Environmental Entomology 5:281-282.

Nealis, V.; Erb, S. 1993. A sourcebook for management of the gypsy moth. Sault Ste. Marie, Ontario: Forestry Canada, Ontario Region, Great Lakes Forestry Centre; 48 p.

Onken, A. 1996. Evaluation of a ready-to-use formulation of Gypchek against gypsy moth populations in Michigan and Virginia. M.S. thesis; 86 p.

Podgwaite, J. 1991. Gypchek production *in vivo*. Proceedings of the U.S. Department of Agriculture Interagency Gypsy Moth Research Forum. 1991. p. 44. USDA Forest Service General Technical Report NE-167, Radnor, PA.

Podgwaite, J.; Dubois, N.; Reardon, R.; Witcosky, J. 1993. Retarding outbreak of low-density gypsy moth populations with aerial applications of Gypchek and *Bacillus thuringiensis*. J. Economic Entomology 86:730-734.

Podgwaite, J.; Reardon, R.; Walton, G.; Venables, L.; Kolodny-Hirsch, D. 1992a. Effects of aerially applied Gypchek on gypsy moth populations in Maryland woodlots. J. Economic Entomology 85:1136-1139.

Podgwaite, J.; Reardon, R.; Walton, G.; Witcosky, J. 1992b. Efficacy of aerially applied Gypchek against gypsy moth in the Appalachian highlands. J. Entomological Science 27:337-344.

Podgwaite, J.; Shapiro, M. 1986. Evaluation of sunlight protectants for gypsy moth nucleopolyhedrosis virus. *In:* Fundamental and applied aspects of invertebrate pathology. Proceedings of the Fourth International Colloquium of Invertebrate Pathology, R.A. Samson, J.M. Vlok and D. Peterson, Eds., Wagemingen, The Netherlands, pp 154-160.

Podgwaite, J.; Shields, K.; Zerillo, R.; Bruen, R. 1979. Environmental persistence of the nucleopolyhedrosis virus of the gypsy moth. Environmental Entomology 8:523-536.

Raimo, B.; Reardon, R.; Podgwaite, J. 1977. Vectoring gypsy moth nucleopolyhedrosis virus by *Apanteles melanoscelus*. Entomophaga 22:207-215.

Reardon, R. 1991. Appalachian gypsy moth integrated pest management project. Forest Ecology and Management 39:107-112.

Reardon, R.; McManus, M.; Kolodny-Hirsch, D.; Tichenor, R.; Raupp, M.; Schwalbe, C.; Webb, R.; Meckley, P. 1987. Development and implementation of a gypsy moth integrated pest management program. J. Arboriculture 13:209-216.

Reardon, R.; Podgwaite, J. 1976. Disease-parasitoid relationships in natural populations of *Lymantria dispar* in the Northeastern United States. Entomophaga 21:333-341.

Reardon, R.; Podgwaite, J. 1994. Summary of efficacy evaluations using aerially applied Gypchek against gypsy moth in the U.S.A. J. Environmental Science and Health B(29): 739-756.

Slavicek, J. 1995. Development of enhanced viral strains for cell culture production. Proceedings U.S. Department of Agriculture Interagency Gypsy Moth Research Forum 1995. p. 113. USDA Forest Service General Technical Report NE-213, Radnor, PA.

Slavicek, J.; Podgwaite, J. 1991. Analysis of *Lymantria dispar* nuclear polyhedrosis viruses (LdNPV) isolated from Gypchek: Purification of high potency LdNPV isolates. Proceedings of the U.S. Department of Agriculture Interagency Research Forum 1991. p. 47. USDA Forest Service General Technical Report NE-167, Radnor, PA.

U.S. Department of Agriculture. 1995. Gypsy Moth Management in the United States: a cooperative approach. Final environmental impact statement Vol. 1-5. Radnor, PA: USDA-Forest Service and Animal and Plant Health Inspection Service; [pagination not continuous].

Webb, R.; Dill, N.; McLaughlin, J.; Kershaw, L.; Podgwaite, J.; Cook, S.; Thorpe, K.; Farrar, R. Jr.; Ridgway, R.; Fuester, R.; Shapiro, M.; Argauer, R.; Venables, L.; White, G. 1996. Blankophor BBH as an enhancer of nuclear polyhedrosis virus in arborist treatments against the gypsy moth (Lepidoptera: Lymantriidae).

Webb, R.; Dill, N.; Podgwaite, J.; Shapiro, M.; Ridgway, R.; Vaughn, J.; Venables, L.; Argauer, R. 1994a. Control of third and fourth instar gypsy moth (Lepidoptera: Lymantriidae) with Gypchek combined with a stilbene disulfonic acid additive on individual shade trees. J. Entomological Science 29:82-91.

Webb, R.; Podgwaite, J.; Shapiro, M.; Tatman, K.; Douglass, L. 1990. Hydraulic spray application of Gypchek as a homeowner control tactic against gypsy moth (Lepidoptera: Lymantriidae). J. Entomological Science 25: 383-393.

Webb, R.; Shapiro, M.; Podgwaite, J.; Lynn, D.; Dougherty, E.; Ridgway, R.; Venables, L.; Cohen, D. 1993. Field comparisons of doses and strains of gypsy moth nuclear polyhedrosis virus against gypsy moth (Lepidoptera: Lymantriidae) in western Maryland in 1990. J. Economic Entomology 86:1185-1190.

Webb, R.; Shapiro, M.; Podgwaite, J.; Reardon, R.; Tatman, K.; Venables, L.; Kolodny-Hirsch, D. 1989. Effect of aerial spraying with Dimilin, Dipel, or Gypchek on two natural enemies of the gypsy moth. J. Economic Entomology 82:1695-1701.

Webb, R.; Shapiro, M.; Podgwaite, J.; Ridgway, R.; Venables, L.; White, G.; Argauer, R.; Cohen, D.; Witcosky, J.; Kester, K.; Thorpe, K. 1994b. The effect of optical brighteners on the efficacy of gypsy moth (Lepidoptera: Lymantriidae) nuclear polyhedrosis virus in forest plots with high or low levels of natural virus. J. Economic Entomology 87:134-143.

Woods, S.; Elkinton, J. 1987. Bimodal patterns of mortality from nuclear polyhedrosis virus in gypsy moth populations. J. Invertebrate Pathology 50:151-157.

Appendix A

Label

PRECAUTIONARY STATEMENTS
HAZARD TO HUMANS

WARNING

Causes eye irritation. Do not get in eyes.

FIRST AID

In case of eye contact, immediately flush eyes with plenty of water for at least 15 minutes. If eyes become irritated, call a physician.

ENVIRONMENTAL HAZARDS

Avoid application to lakes, streams, or ponds. Do not contaminate water by cleaning of equipment or disposal of wastes.

DIRECTIONS FOR USE
GENERAL CLASSIFICATION

It is a violation of Federal law to use this product in a manner inconsistent with its labeling.

For foliar protection from gypsy moth larvae make 1 application of at least 400 billion gypsy moth polyhedral inclusion bodies per acre, or make 2 or more applications 2 to 4 days apart at the rate of 200 to 500 billion gypsy moth polyhedral inclusion bodies per acre, in sufficient spray formulation for thorough and uniform coverage. Stickers and u.v. protectants may enhance performance of this product. Refer to technical bulletin for mixing and application instructions. **NEVER USE CHLORINATED WATER IN THE SPRAY FORMULATION.**

STORAGE AND DISPOSAL

Activity may be impaired by storage above 90° F.

Do not contaminate water, food or feed by storage or disposal. Open dumping is prohibited.
Do not reuse empty container.

Pesticide, spray mixture, or rinsate that cannot be used should be disposed of in a landfill approved for pesticides or buried in a safe place away from water.

Container disposal: Triple rinse and dispose of in an approved landfill or bury in a safe place.

Consult Federal, State or local disposal authorities for approved alternative procedures.

GYPCHEK
BIOLOGICAL INSECTICIDE FOR THE GYPSY MOTH

Active Ingredient:*
Polyhedral inclusion bodies of gypsy moth
nucleopolyhedrosis virus 14.6%
Inert ingredients 85.4%
TOTAL 100.0%

*This lot contains at least _____ billion gypsy moth polyhedral inclusion bodies per gram.

KEEP OUT OF REACH OF CHILDREN

WARNING

See back of tag for additional precautionary statements.

For use in only wide-area government sponsored pest control programs.

NOTICE: The U.S. Forest Service makes no warranty, express or implied including the warranties of merchantability and/or fitness for any particular purpose, concerning this material except those which are contained on the U.S. Forest Service's label.

MFG. BY: U.S. Forest Service, USDA
P.O. Box 2417
Washington, D.C. 20013

EPA ESTABLISHMENT NO. 27586-CT1

EPA REGISTRATION NO. 27586-2

NET WEIGHT: _____ **LOT NO.:** _____

Appendix B

Material Safety Data Sheet

MATERIAL SAFETY DATA SHEET[1]

(Form "Essentially similar" to OMB No. 1218-0072)

PRODUCT NAME: GYPCHEK	CHEMICAL NAME: Nucleopolyhedrosis Virus of *Lymantria dispar*, The Gypsy Moth
FORMULA: As a biological organism, this A.I. does not lend itself to characterization by normal pesticide criteria.	MOLECULAR WEIGHT: N/A
SYNONYMS: N/A	CHEMICAL FAMILY: N/A

I. PHYSICAL DATA

BOILING POINT: N/A	FREEZING POINT: N/A
SPECIFIC GRAVITY ($H_2O=1$): N/A	VAPOR PRESSURE (mm Hg): N/A
VAPOR DENSITY (AIR=1): N/A	EVAPORATION RATE (Butyl Acetate=1): N/A
%VOLATILES BY VOLUME: N/A	SOLUBILITY IN WATER (% by wt): INSOLUBLE

APPEARANCE AND ODOR:
Dried Insect body parts and virus polyhedra

11. HAZARDOUS INGREDIENTS

MATERIAL	PERCENT	TLV (Units)
N/A		

III. FIRE AND EXPLOSION HAZARD DATA

FLASH POINT (METHOD USED): N/A	FLAMMABLE LIMITS N/A

EXTINGUISHING MEDIA:
Water on burning container.

SPECIAL FIRE FIGHTING PROCEDURES:
None except to avoid inhalation of particulates released by fire.

UNUSUAL FIRE AND EXPLOSION HAZARDS:
N/A

1 Prepared in compliance with the U.S. Department of labor, Occupational Safety and Health Administration (OSHA) Hazard Communication Standard (29 CFR 1910.1200)

IV. HEALTH HAZARD DATA

THRESHOLD LIMIT VALUE:	Not established
EFFECTS OF OVEREXPOSURE:	None known.
EMERGENCY AND FIRST AID PROCEDURES:	Remove from exposure situation.
NOTES TO PHYSICIAN:	Allergies and hypersensitivity may occur in some individuals.

V. REACTIVITY DATA

STABILITY: UNSTABLE ☐ STABLE ☐

CONDITIONS TO AVOID	Do not store in sunlight at high temperatures.
INCOMPATIBILITY (materials to avoid)	N/A
HAZARDOUS DECOMPOSITION PRODUCTS	N/A

HAZARDOUS POLYMERIZATION: MAY OCCUR ☐ WILL NOT OCCUR ☐

CONDITIONS TO AVOID	None

VI. SPILL OR LEAK PROCEDURES

STEPS TO BE TAKEN IF MATERIAL IS RELEASED OR SPILLED	Containment and cleanup by placing in a sealable container for transport to an approved landfill or other disposal site.
WASTE DISPOSAL METHOD	Burn bags or triple rinse containers and dispose them and excess product at an appropriate site.

VII. SPECIAL PROTECTION INFORMATION

RESPIRATORY PROTECTION (Specify type)	Use normal clothing and medical face mask as appropriate.
VENTILATION: LOCAL EXHAUST	N/A
MECHANICAL (general)	N/A
PROTECTIVE CLOTHING:	Goggles and face mask as appropriate.
OTHER PROTECTIVE EQUIPMENT:	None except to have eyewash, soap and water available.

VIII. SPECIAL PRECAUTIONS

PRECAUTIONARY LABELING	Keep out of reach of children For use only in USDA-FS-supervised projects. Avoid direct application to water. Do not contaminate water by inappropriate disposal.
OTHER HANDLING AND STORAGE CONDITIONS	Avoid heat and sunlight. Dispose in approved sanitary landfill or by incineration, as allowed by State and local authorities. Do not reuse "empty" containers.

DEPARTMENT OF TRANSPORTATION	HAZARD CLASSIFICATION	Non-hazardous, non-toxic
	SHIPPING NAME	Nucleopolyhedrosis Virus
EPA REGISTRATION #	27586-2	
NAME	Nucleopolyhedrosis Virus	

The USDA Forest Service (FS) believes that the data contained herein are factual and the opinions expressed are those of qualified experts regarding the results of the tests conducted The data are not to be taken as a warranty or representation for which the FS assumes legal responsibility. They are offered solely for your cooperation, investigation, and verification. Any use of these data and information must be determined by the user to be in accordance with applicable Federal, State and local laws and regulations.

USDA Forest Service
P.O. Box 96090
Washington, DC 20090

Emergency Telephone No. (202) 205-1107

Appendix C

Technical Bulletin

TECHNICAL BULLETIN

GYPCHEK BIOLOGICAL INSECTICIDE FOR THE GYPSY MOTH*

Gypchek Biological Insecticide consists of occlusion bodies (OBs) (polyhedra) of the gypsy moth nucleopolyhedrosis virus. Care must be taken in the storage, mixing and application of the technical powder. The virus is very sensitive to sunlight and temperatures above 55° C (131°F) and should be stored under refrigeration and in the dark prior to use. Temperatures below freezing are recommended for long term storage. Since Gypchek is especially active against smaller larvae, it is recommended that applications be made as soon as hatch is complete, all larvae are off the egg masses and actively feeding, and the majority of larvae are in the late-first instar. Further, oak leaves, or other target foliage, should be at least 20% expanded at the time of spray.

MIXING AND APPLICATION FOR AERIAL TREATMENTS

A spray adjuvant (Carrier 038, Abbott Laboratories) has been specifically developed for use with Gypchek. Carrier 038 possesses sunlight-shielding and antievaporative properties superior to a standard lignosulfonate-molasses tank-mix that is also used with Gypchek. The two tank-mixtures described below are for aerial application and should be applied in sufficient volume for thorough and uniform foliar coverage. The current recommendation for the Carrier 038 tank-mix is two applications, two to four days apart, at the rate one gallon (U.S.) finished spray per acre for each application. The current recommendation for the lignosulfonte-molasses tank-mix is two applications, two to four days apart, at the rate of two gallons (U.S.) finished spray per acre for each application. Use boom and nozzle systems or rotary atomizers designed to result in droplets 100-450μm $D_{V.5}$(=VMD)** (For example: Beecomist 275, Micronair AU-5000, or flat fan 8004, 8008). It is recommended that applications be made early in the morning and not be made if rain is predicted within 24 hrs.

CARRIER 038 TANK MIXTURE (PER GALLON)

Gypchek	200-500 billion OBs
Carrier 038	0.95 gallon (122 fl. oz.)
Water	0.05 gallon (6 fl. oz.)

The finished tank-mix should be prepared 95 parts Carrier 038 to 5 parts Gypchek-water slurry (vol/vol) (For example: a 3 gallon slurry of Gypchek would be used with 55 gallons of Carrier 038 to treat 58 acres)

Important: Check pH of water from field source. If pH exceeds 7.5 or is below 5.5, add sufficient acid or base to adjust ***pH to approximately 7. NEVER USE CHLORINATED WATER TO PREPARE THE GYPCHEK SLURRY.

MIXING INSTRUCTIONS

1. Depending upon the amount of tank-mix necessary, prepare a Gypchek water slurry, either in a clean pail, or in the mix tank with circulation. Mix until the powder is evenly dispersed and there are no clumps. Whenever practical, shield the powder from direct sunlight while preparing the slurry. **DO NOT ADD POWDER DIRECTLY TO AIRCRAFT HOPPER**

2. If the Gypchek-water slurry has been prepared in the mix-tank...Slowly add the required amount of Carrier 038 with agitation. Continue to mix thoroughly for 15-25 minutes before loading aircraft.

3. If the Gypchek-water slurry has been prepared in a pail...don't pour it directly into an empty mix-tank, rather, add about 10 gallons of Carrier 038 to the tank and start agitation. Then, pour the Gypchek-water slurry into the tank. Rinse the pail with a small amount of water and add the rinsate to the tank. Agitate for a few minutes and then slowly add the rest of the Carrier 038. Continue to mix thoroughly for 15-20 minutes before loading aircraft.

LIGNOSULFONATE-MOLASSES TANK MIXTURE (PER GALLON)

Gypchek	100-250 billion OBs
Lignosite® AN (Georgia Pacific)	0.5 lb.
Feed-grade molasses.	0.13 gallon (16 fl. oz.)
Bond® (Loveland Industries)	0.02 gallon (2.5 fl. oz.)
Water	0.85 gallon (110 fl. oz.)

MIXING INSTRUCTIONS

1. Fill mix tank with the amount of <u>nonchlorinated</u> water (pH<7.5.)*** necessary for the desired acreage. While circulating, slowly add the necessary Lignosite AN powder.

2. When the Lignosite AN is in solution add the molasses and mix thoroughly for about 5 minutes. The lignosulfonate AN-molasses mix can stand overnight, perhaps two nights if cool.

3. Just before spray add Bond, then Gypchek. The Gypchek powder should be added slowly to avoid clumping. Gypchek can also be added as a slurry described above. Circulate for 15-20 minutes and load as per aircraft specifications.

MIXING AND APPLICATION FOR GROUND TREATMENTS

The tank mixture below is for ground application using hydraulic equipment and should be applied in sufficient volume for thorough and uniform foliar coverage. For spraying roadsides, woodlots or small acreages, it is recommended that one application be made at the rate of 100 gallons (U.S.) finished spray per acre. For individual trees in homeowner situations it is recommended that trees be sprayed once to runoff (For example: 15-25 gallons for large oaks).

LIGNOSULFONATE TANK MIXTURE (PER GALLON)

Gypchek	10 billion OBs
Lignosite® AN	0.5 lb.
Bond®	0.02 gallon (2.5 fl. oz.)
Water	0.98 gallon (125 fl. oz.)

MIXING INSTRUCTIONS

1. Fill hydraulic sprayer mix tank with the amount of nonchlorinated water (pH<7.5.)*** necessary for the desired acreage. While circulating, slowly add the necessary Lignosite AN powder.

2. When the Lignosite AN is in solution add Bond, then Gypchek, either as a powder or a slurry. Circulate for 15-20 minutes before spraying.

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* Read label thoroughly before using and follow all cautions and directions.

** The droplet size that divides the spray volume in half; 50% of the droplets are above the $D_{V.5}$ and 50% are below.

*** Use products that are available for adjusting the pH of swimming pool water.

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